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Influence of population isolation on genetic variation and demography in Seychelles warblers: a field experiment

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Summary

Between 1959 and 1968 the entire world population of Seychelles warblers (*Acrocephalus sechellensis*) comprised only 26–29 individuals entirely confined to Cousin Island (26 ha). Through conservation actions the island population has reached a carrying capacity of *ca* 320 birds since 1982. The warbler population has significantly lower levels of genetic variation, as determined by multilocus DNA fingerprinting, compared with its mainland relative, the Australian warbler (*Acrocephalus australis*) and other outbred natural bird populations (mean band sharing coefficients are 0.50, 0.22 and 0.21, respectively), and has a very low annual reproductive rate of 0.28 yearlings per breeding pair. Therefore some degree of inbreeding may have occurred in this population.

The relatively low level of genetic variation is not directly responsible for this low reproductive output since breeding pairs that were transferred to the islands of Aride (68 ha) and Cousine (29 ha) with similar vegetation and climate, but with higher year-round insect food abundance, had significantly higher fecundity and survival. In contrast, no difference in reproductive success was observed between incestuous breeding pairs and non-incestuous breeding pairs.

In addition, annual production of yearlings by pairs on Aride was on average 44 times higher than that by the *same* pairs on Cousin before the transfer. Annual production of yearlings by pairs on Cousine, that were as inbred as those on Aride, was on average five times higher than that by the *same* pairs on Cousin before the transfer. Eight years after translocation, the Aride population had grown to over 1000 birds, and six years after translocation, the Cousine population had grown to 186 birds.

The experiments, controlled for group size, breeding partner, breeder age and experience, showed that the differences in reproductive success by warblers on the three islands cannot be due to genetic differences because of similar band-sharing similarities, but seem entirely accounted for by differences in food supply. Although the amount of genetic variation in the Seychelles warblers is relatively low, the demographic parameters do not indicate apparent signs of inbreeding depression in these populations.

INTRODUCTION

The genetic and demographic consequences of population fragmentation and isolation are of major interest for conservation biology (Soulé, 1986). In small populations loss of genetic variation and an increase in homozygosity due to inbreeding and the stochastic effects of genetic drift, are thought to reduce the fitness of a population (Lerner, 1954; Soulé, 1987; Charlesworth & Charlesworth, 1987). Studies of genetic variation in small populations of different species provide opportunities to address the effects of microevolutionary processes pertaining to population differentiation and to conservation genetics. An analysis of the effects of loss of genetic variation and inbreeding on population viability is necessary for the understanding of long-term persistence of endangered populations and the evaluation of their extinction rates. Small, inbred populations may have lowered fecundity (Ralls & Ballou, 1983; Gilpin & Soulé, 1986), a higher susceptibility to infectious agents (O'Brien *et al.*, 1987), and consequently lower survival rates (Franklin & Soulé, 1981;

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Schonewald-Cox *et al.*, 1983; Soulé, 1987; Hedrick & Miller, 1992; Bijlsma, Ouborg & van Treuren, 1994). The lack of genetic variation may limit a population's ability to respond to changes in environmental conditions (Gilpin & Soulé, 1986; Lande & Barrowclough, 1987). A high degree of inbreeding and low levels of genetic variation can be the result of one or more bottlenecks. Important determinants are the number of reproducing individuals during a bottleneck period and the length of the bottleneck period (Nei, Maruyama & Chakraborty, 1975). We tested the viability of two small populations of the cooperatively breeding Seychelles warbler (*Acrocephalus sechellensis*), that were derived from the same natural, inbred population and that showed similar levels of genetic variation, upon transfer to two separate islands with comparable environmental conditions.

Between 1959 and 1968 the entire world population of the Seychelles warbler has experienced a narrow bottleneck of 26–29 birds confined to Cousin Island (29 ha) in the Seychelles (Crook, 1960). Following long-term management of Cousin by Birdlife International since 1968, the warbler population made a spectacular recovery with numbers rising to 320 birds in 1982 (Fig. 1). Since then, the population has not increased, but fluctuated around that level (Fig. 1; Komdeur, 1994*a*). Although warblers can breed successfully in their first year, some individuals remain on their natal territories as helpers, providing care in the rearing of their parents' further offspring (Komdeur, 1994*b*). The breeding success of parents is significantly affected by both the presence of helpers (Komdeur, 1994*c*) and territory quality (Komdeur, 1996*a*). Given the vulnerability of one small island population, 29 birds (16 males and 13 females) were translocated successfully from Cousin Island to Aride Island (68 ha) in 1988, and another 29 birds to Cousine Island (26 ha) in 1990, in order to establish additional warbler populations (Komdeur, 1994*a*).

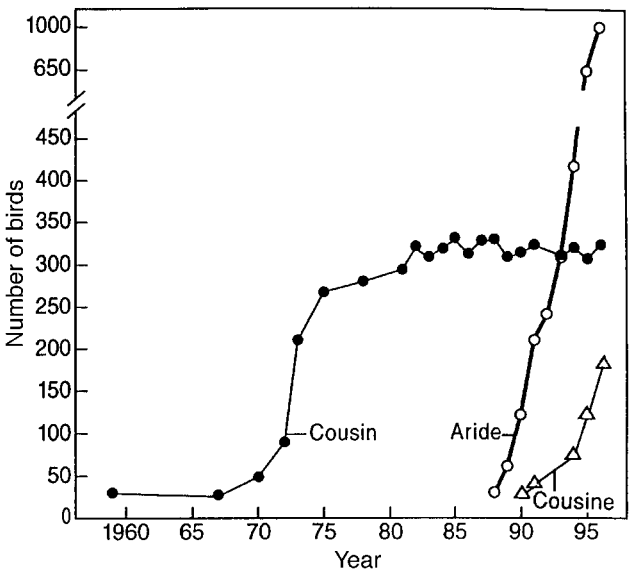

Fig. 1. Increase in Seychelles warbler numbers on Cousin Island since 1959, on Aride Island since September 1988, and on Cousine Island since 1990.

In this paper we compare the genetic variation as determined by multilocus DNA fingerprinting (Jeffreys, Wilson & Thein, 1985) of these three warbler populations. We further discuss whether the low reproductive output of the Cousin Island population can be associated with low levels of genetic variation or reduced food availability. We use 15 years of data from a genealogically known population. The transfers of inbred warbler pairs from Cousin Island to the previously unoccupied islands of Aride and Cousine with significantly more food available year-round allowed us to compare the reproductive output of the same pairs pre- and post-transfer, under the same climatic conditions.

MATERIALS AND METHODS

Study area and data collection

The entire population of Seychelles warblers on Cousin Island (115–123 breeding groups, 310–400 birds) was under continuous study between January 1982 and August 1996. Between 23 and 29 September 1988, 29 colour-ringed adult warblers (16 males and 13 females; all between three and five years old) were caught in 13 randomly distributed territories on Cousin Island and transferred to Aride Island by motor-boat. During a three-day period from 29 June to 1 July 1990, 29 colour-ringed adult warblers (15 males and 14 females) were transferred from Cousin to Cousine. All of the warblers on the island of Cousin and Aride were studied simultaneously from September 1988 to August 1996; on Cousine they were studied from June 1990 to August 1996. On Aride, 16–49 breeding groups (29–300 birds) were studied, and on Cousine, 12–33 groups (29–138 birds). Data presented here are based on individually colour-ringed birds.

Breeding activity

The breeding activity and the proportion of birds that survived were recorded by monitoring all territories on Cousin, Aride and Cousine every two weeks for nests with clutch or young and for the presence of colour-ringed birds. Once a bird was missing from its territory, all other territories were checked to assess dispersal. As Seychelles warblers have never colonized other islands naturally, we assumed that missing birds had died if they were not found on other territories. All nestlings were individually colour-ringed. The effect of habitat quality on reproductive success in warblers was studied by comparing the annual breeding performance of breeding pairs comprising unrelated birds occupying high- and low-quality territories on Cousin Island (1990–1991); for territory quality see below. The effect of inbreeding on reproductive success was determined by comparing the annual breeding performance of breeding pairs comprising unrelated birds and incestuous breeding pairs (matings between parent–offspring, and brother–sister) occupying high quality territories on Cousin Island (1990–1991). Kinship calculations were based on genealogical data.

The three islands have the same climate and vegetation types (Komdeur, 1996*a*) and are only 12 km apart, so we employed matched pairs statistical tests to compare breeding activity and climatical data between them. Group size effects on reproduction were isolated by comparing only warbler pairs without helpers. In order to determine the effect of food availability on breeding performance on Aride and Cousine, breeding pairs with known band-sharing similarity were used, comprising original Cousin birds only.

In 1993–1994, warblers of known hatching dates and genealogical history were captured with mist nets to take blood samples for use in DNA fingerprinting. In 1993, 30 birds were sampled on Cousin Island of which 21 were alive at the time of transfer to Aride in 1988 and 24 were alive at the time of transfer to Cousine in 1990. In 1993, 29 birds were sampled on Aride, including four breeding pairs and five single individuals that had been translocated from Cousin Island. In 1994, 26 birds were sampled on Cousine, including four breeding pairs and eight single individuals that had been translocated from Cousin Island. To strengthen the outcome of our analyses, we compared the band-sharing coefficient of the Seychelles warbler with that of unrelated birds from another *Acrocephalus* species. Since 1997 the endemic continental Australian warbler (*Acrocephalus australis*), which is widely distributed all over Australia, has been studied alongside the Seychelles warbler. In 1997, 34 warblers were caught, colour-ringed and bloodsampled at Edithvale, Melbourne, Australia. All bloodsamples (*ca* 50 µl) were caught from the brachial vein and placed in 1 ml of DMSO buffer (25% dimethyl sulphoxide in 4 M NaCl) and stored at 4°C.

Territory quality, island food abundance and weather records

Territory and island quality, independent of the presence of nest sites and nest predators (Komdeur, 1991, 1994*a, c*), were measured in terms of the insect prey abundance, because this correlated with adult survival and reproductive success (Komdeur, 1991). The Seychelles warblers are insectivorous, and so territory quality was expressed as mean number of prey invertebrates available within a territory (for method, see Komdeur, 1991, 1994*a*) and island quality as prey invertebrates present per site (for method, see Komdeur, 1996*a*). The quality of territories on Cousin was assessed monthly from May 1987 to June 1991, simultaneously on Aride from September 1988 to September 1990, and simultaneously on Cousine from June 1990 to June 1991). The quality of breeding territories remained constant between years (Komdeur, 1996*a*), so territory quality was expressed as the average quality over the whole research period. Each month, island quality of Cousin and Aride was studied from May 1987 to September 1990, and every three months from November 1990 to November 1991; island quality of Cousine was studied from August 1989 to September 1990 and every three months from November 1990 to November 1991. Island quality was determined by monitoring invertebrate prey abundance simultaneously on Cousin, Aride and Cousine at 67, 28 and 20 randomly chosen sites, respectively.

Daily weather records were kept of rainfall and maximum day temperature. Rainfall frequency was the number of days per month (corrected to a 30 day month) that 0.1 mm or more rainfall was recorded.

Removal experiments

The translocations of Seychelles warblers, and the fact that some breeding pairs from Cousin were bloodsampled and remained together on the new islands, enabled us to measure the influence of food availability and degree of genetic variation on breeding success by comparing pre-transfer and post-transfer data for each pair. The experimental units consisted of four bloodsampled Cousin pairs removed to Aride (Experiment 1) and four bloodsampled Cousin pairs removed to Cousine (Experiment 2). The control units for both translocations each consisted of five different bloodsampled breeding pairs on Cousin. The annual reproductive success of each pair was known exactly in the two years before and two years (Experiment 1) or one year after (Experiment 2) the translocation. The outcome of Experiment 2 created an appropriate replicate test of Experiment 1.

All the pairs were experienced breeders because they had bred successfully (i.e. raised fledglings) in two consecutive years before transfer. During the whole experimental period, no helpers had assisted the breeding pairs in raising broods, the breeding pair remained the same, and the ages of breeding birds remained within the three to seven year age interval, that is, the period during which annual reproductive success remained constant for both breeding males and females (Komdeur, 1996*b*). Therefore, the internal control used in this study (comparing reproduction pre- and post-transfer of the same breeding pairs) is an explicit matched-pairs control for group size, age and experience of the breeding pairs.

DNA fingerprinting, inbreeding and genetic variation

Blood samples were processed for DNA fingerprinting as described in Kappe, Komdeur *et al.* (1998). Blood cells were lysed by proteinase K treatment, DNA was purified by phenol extractions and precipitated with ethanol. Five micrograms of DNA were digested to completion with *Hae*III. ^{32}P-dCTP-labelled (Feinberg & Vogelstein, 1983) Jeffrey's probe 33.15 was used to generate fingerprint patterns on Southern blots of the digested DNA. Band-sharing coefficient was calculated by comparing fingerprints of similar intensity run in adjacent lanes (Fig. 2). Within the range of 3–20 kb a total of 37 informative bands were resolved. All clear bands within this range were scored. The coefficient of band-sharing between individuals was calculated as $S_{xy} = 2n_{xy}/(n_x + n_y)$, where n_x and n_y are the number of bands present in individuals x and y, respectively, and n_{xy} is the number of bands shared by x and y (Wetton, Carter *et al.*, 1987). Mean

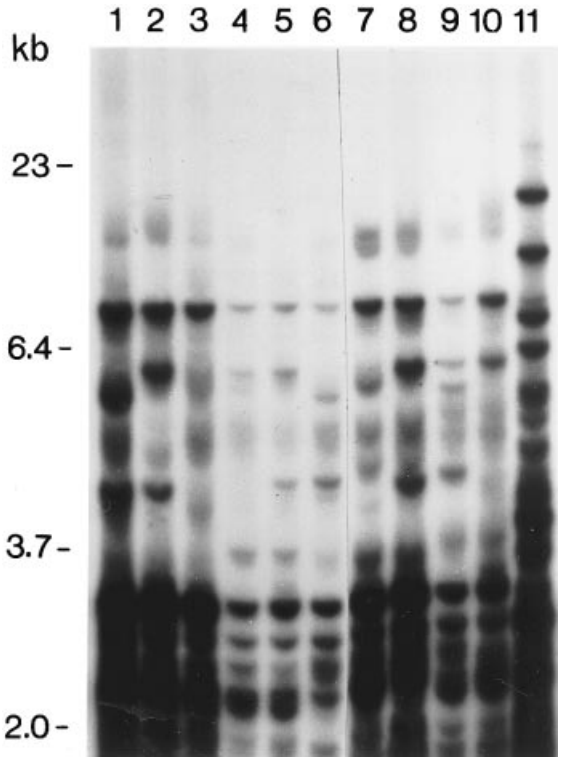

Fig. 2. Example of DNA fingerprints of 11 Seychelles warblers on Cousin Island. Fingerprints obtained with probe 33.15. Kilobase scales were inferred from lanes containing 1.5 µg lambda DNA digested with *Bst*E_2.

population similarity was estimated by averaging the similarities of all pairs of individuals in the sample. The standard error of the mean similarity was estimated by the method described by Lynch (1990), which considers variances and covariances between similarity indices. All overlapping pairs of individuals were used to estimate the sampling variance. Covariance of overlapping similarities was estimated using all possible pairwise combinations sharing one individual (Kappe, Bijlsma *et al.*, 1997). Similarity of band-sharing between populations, corrected for within-population similarity, was estimated using Lynch's (1991) formula, $S_{ij} = 1 + S'_{ij} - [S_i + S_j)/2]$, where S_i is the mean proportion of bands shared among individuals of population i, and S'_{ij} is the mean proportion of bands shared between random pairs of individuals across populations i and j.

Unless stated otherwise, means are expressed with standard deviations, probability values are two-tailed, and the null hypothesis was rejected at $P < 0.05$.

RESULTS

Seasonal food abundance on the islands of Cousin, Aride and Cousine

Between May 1987 and November 1991 peak food supplies on Cousin occurred once a year between June and November. During the same period the mean food abundance per site on Aride was on average 3.4 times higher than that on Cousin (mean: 191.6 ± 85.7 *versus* 56.2 ± 38.4; $n = 47$; paired-sample t test: $t = 13.90$, d.f. = 46, $P < 0.001$) (Fig. 3). The maximum recorded food abundance for Cousin was slightly higher than the minimum recorded on Aride. From August 1989 to November 1991, parallel studies showed that food abundance on Cousine was on average 1.8 times higher than that on Cousin (mean: 102.0 ± 68.9 *versus* 57.6 ± 46.1; $n = 20$; paired-sample t test: $t = 5.51$, d.f. = 19, $P < 0.001$) but 0.6 times as high as that on Aride (mean: 177.0 ± 92.1; $n = 20$; paired-sample t test: $t = 6.09$, d.f. = 19, < 0.001) (Fig. 3).

Reproductive rate and genetic variation of warblers on Cousin island

The Seychelles warbler on Cousin has a low reproductive rate. Between 1986 and 1991, peaks of nest-building occurred only once a year, during a short period (Fig. 4). The majority of nests were built between May and August. The average clutch size was 1.09 ± 0.34 (n = 223). The maximum clutch laid by a single female was two eggs, but 91.0% of clutches contained a single egg. Warbler pairs produce on average 0.28 ± 0.21 young per year that survive to one year of age (n = 334). Warblers are long-lived: the mean annual adult survivorship (the probability of surviving to the next year, starting at age one year old) is 81.5% (n = 239 bird years) and mean adult life expectancy is 4.4 years. Because birth and death rate are in equilibrium, the warbler population on Cousin is stable, and assumed to have reached the carrying capacity (Fig. 1).

It seems that different levels of inbreeding have no effect on reproductive success in the warblers investigated (Table 1). The breeding performances of incestuous pairs comprising parent–offspring and brother–sister breeder combinations were the same as that of non-incestuous pairs within the same habitat: they attempted the same number of nests, produced the same number of nests with clutch and nestlings, and the same number of nests resulted in fledglings and yearlings. Although pairs occupying high-quality territories produced a similar number of nesting attempts as the pairs occupying low-quality territories, the amount of insect food present in a territory seems to be an important factor for further reproductive success (Table 1). The significantly better reproduction by pairs on high-quality territories was attributed to more nests resulting in a clutch, and higher survival of clutches to hatching. On average pairs on high-quality territories produced 7.3 times more yearlings per year than pairs on low-quality territories.

In 1988, at the time of transfer to Aride, the average band-sharing coefficient (± SE) within the Seychelles warbler population on Cousin was 0.51 ± 0.01 (n = 34); in 1990, at the time of transfer to Cousine, 0.49 ± 0.01 (n = 40); and in 1993, 0.50 ± 0.01 (n = 30). These values are significantly higher than that for the Australian warbler population in Edithvale, Melbourne. The average band-sharing coefficient within the Australian warbler population was 0.22 ± 0.01 (n = 34). The Seychelles

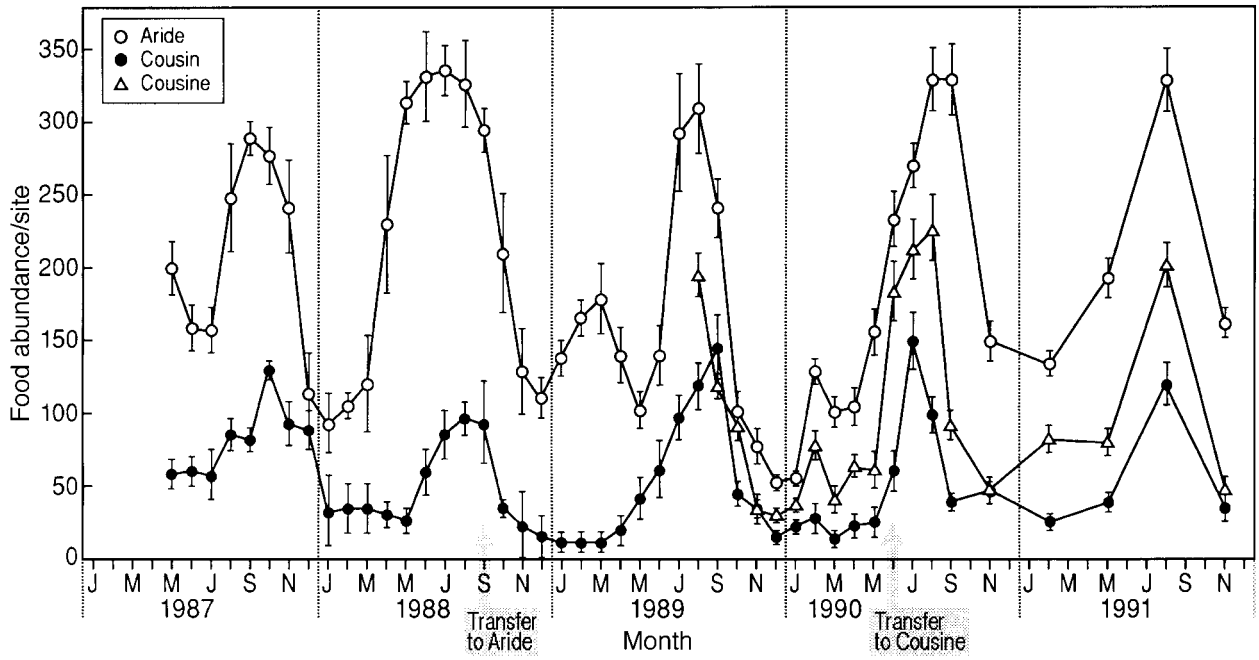

Fig. 3. Monthly food abundance (± SE) of the islands of Aride, Cousin and Cousine (January 1987–November 1991).

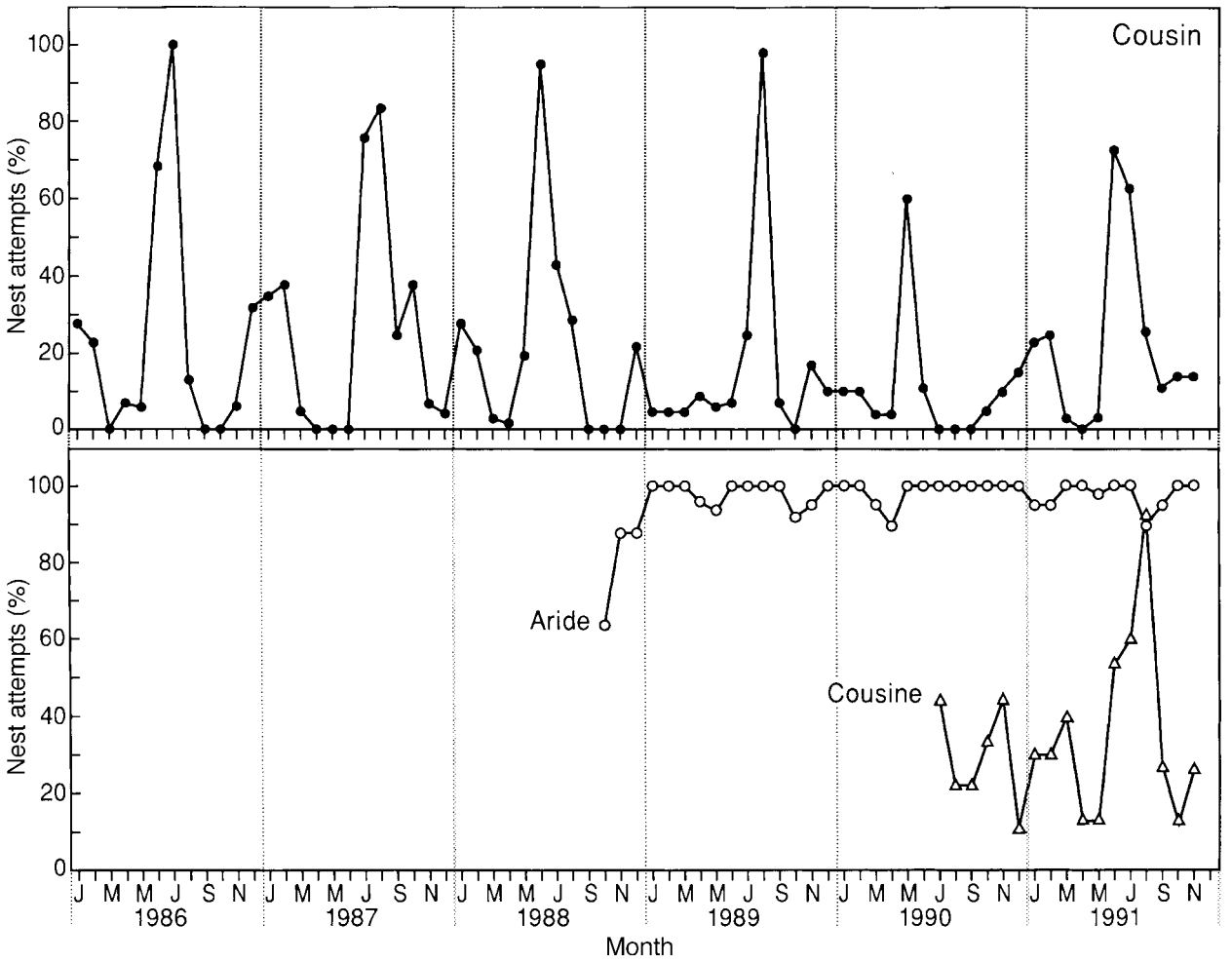

Fig. 4. Percentage of territories with nest attempts each month on the islands of Cousin (January 1986–November 1991), Aride (October 1988–November 1991) and Cousine (July 1990–November 1991).

Table 1. Influence of relatedness between breeders and territory quality on annual breeding performance (± SD) of Seychelles warbler pairs on Cousin Island (1990–1991)

	High quality territory			Low quality territory	*P*			
	Unrelated pair 1	Parent × offspring 2	Brother × sister 3	Unrelated pair 4	1 *vs* 2	1 *vs* 3	2 *vs* 3	1,2,3 *vs* 4
Annual performance/pair								
Nest attempts	1.3 ± 0.5	1.3 ± 0.5	1.3 ± 0.7	1.5 ± 0.7	0.709	1.000	0.781	0.247
Nests with clutch (%)	87.5	100	91.7	46.2	0.684	1.000	1.000	<0.001
Nests with clutch	1.2 ± 0.6	1.3 ± 0.5	1.2 ± 0.7	0.7 ± 0.7	0.737	0.840	0.923	0.005
Clutches hatched (%)	100	90.0	81.8	41.7	0.862	0.357	1.000	<0.001
Nests with nestlings	1.2 ± 0.6	1.1 ± 0.6	1.0 ± 0.5	0.3 ± 0.5	0.881	0.497	0.658	<0.001
Nestlings fledged (%)	85.7	88.9	100	60.0	1.000	0.668	1.000	0.246
Nests producing fledglings	1.0 ± 0.4	1.0 ± 0.8	1.0 ± 0.5	0.2 ± 0.4	1.000	1.000	1.000	<0.001
Fledglings reaching one year of age (%)	83.3	87.5	88.9	66.7	1.000	1.000	1.000	0.958
Nests producing yearlings	0.8 ± 0.5	0.9 ± 0.8	0.9 ± 0.6	0.1 ± 0.3	0.669	0.552	0.969	<0.001
Mean territory quality	38.9 ± 7.4	39.1 ± 5.3	42.0 ± 7.6	6.4 ± 5.0	0.946	0.359	0.385	<0.001
n	12	8	9	17				

Statistical significance of comparisons between annual performance determined by two-tailed *t* test or chi-squared contingency analysis (percentage data)

warbler band-sharing coefficient is of the same order as that reported for other avian species that had experienced a population decline (mean *S*: 0.57 ± 0.03 (n = 4); Brock & White, 1992; Haig, Ballou & Casna, 1994; Haig & Ballou, 1995; Rave, 1995), but much higher than that recorded for outbred natural bird populations (mean *S*: 0.21 ± 0.02 (n = 6); Morton, Forman & Braun, 1990; Westneat, 1990; Wetton, Parkin & Carter, 1992; Yamagishi, Nishiumi & Shimoda, 1992; Dunn & Robertson, 1993; Pinxten *et al.*, 1993). Probably, a substantial level of genetic erosion has occurred in the Seychelles warbler population.

Inter-island comparison of genetic variations and fitness characteristics

At the time of the transfers in 1988 and 1990, the translocated birds were a representative sample of those remaining on Cousin Island. In 1988, the average band-sharing coefficient for the warblers translocated to Aride Island was 0.52 ± 0.01 (n = 13), which was not significantly different from the average band-sharing coefficient of 0.50 ± 0.01 (n = 21) for the warblers remaining on Cousin Island. Similarly, in 1990, the average band-sharing coefficient for warblers translocated to Cousine Island was 0.48 + 0.02 (n = 16), which was not significantly different from the average band-sharing coefficient of 0.49 ± 0.02 (n = 24) for the warblers remaining on Cousin Island. In 1993, the average band-sharing coefficient between the Cousin and Aride Island populations was 0.48 ± 0.06, and in 1993–1994 the average band-sharing coefficient between the Cousin and Cousine Island populations was 0.47 ± 0.07. Both values were similar to the values found within the populations. This implies a between-population similarity of 0.96 and 0.99, respectively.

The transferred birds rapidly accepted their new habitat on Aride and Cousine, forming pairs and establishing territories within days, and in some cases hours, of being released. In most territories, successful nesting started within a few weeks, and in some territories within three days, of release. The first young birds on Aride (twins) and Cousine hatched four and three weeks after the transfer, respectively. The most striking aspect of both transfers was the burst of nesting activity by birds immediately following release and that breeding on Aride occurred outside the 'normal' breeding period on Cousin Island. Comparing the percentage of territories with nest-building activity on Aride and Cousin for the three years and two months after the transfer (Fig. 4), Aride birds showed on average 6.1 times more nest-building activity than Cousin birds (mean monthly nesting activity: 96.7 and 15.8%, respectively; paired sample *t* test: t = 23.92, d.f. = 37, $P < 0.001$). The seasonal pattern in the percentage of territories with nesting activity on Aride was not synchronous with that on Cousin in the same month. A minimum of 64% of pairs bred on Aride in October 1988 and a maximum of 90–100% of pairs were breeding from January 1989 to November 1991. Over the same period the percentage of breeding pairs on Cousin varied from 0% (October and November 1988, and June 1989) to 96% (August 1991 only). Comparing the percentage of territories with nest-building activity on the three islands for one year and five months following the transfer to Cousine (Fig. 4), Cousine birds showed on average 2.9 times more nest-building activity than did Cousin birds (t = 2.92, d.f. = 16, P = < 0.001). There appeared to be one annual peak of breeding activity in the populations on Cousin (June/July) and Cousine (August). The seasonal pattern in the percentage of territories with breeding activity on Cousine was synchronous with that on Cousin in the same month but not with that on Aride.

Annual adult survival (the probability of surviving to the next year, starting at age one year old) measured over the same time period was also significantly higher on Aride compared with Cousin (1988–1990; 100% (n = 65) *versus* 81.9% (n = 249; χ^2 = 12.28, d.f. = 1, $P < 0.0005$), and on Cousine compared with Cousin (1990–1992: 100% (n = 55) *versus* 80.7% (n = 155; χ^2 = 6.97, d.f. = 1, $P < 0.01$).

Within-pair comparison of fitness characteristics before and after transfers

In both experiments, the experimental and control pairs had, based on the degree of similarity, a similar degree of inbreeding. At the time of the transfer to Aride the average band-sharing coefficients of the experimental and control pairs were 0.53 ± 0.08 (n = 8) and 0.48 and 0.09 (n = 10), respectively. The band-sharing coefficient between both groups was 0.50 ± 0.06. At the time of the transfer to Cousine Island, the average band-sharing coefficients of the experimental and control pairs were 0.49 ± 0.05 (n = 8) and 0.48 ± 0.09 (n = 10), respectively. The band-sharing coefficient and similarity between both groups was 0.48 ± 0.06 and 0.97, respectively. During the two-year period before transfer, experimental and control units had the same annual production of yearlings (Fig. 5). After transfer of the experimental groups to the islands of Aride and Cousine, reproductive success of control groups remaining on Cousin did not change significantly (Fig. 5). Furthermore, the reproductive success of both transferred experimental groups was significantly higher than their control groups (Cousin *versus* Aride: $F_{1,14} = 207.24$, $P < 0.001$; Cousin *versus* Cousine: $F_{1,14} = 9.30$, $P < 0.01$).

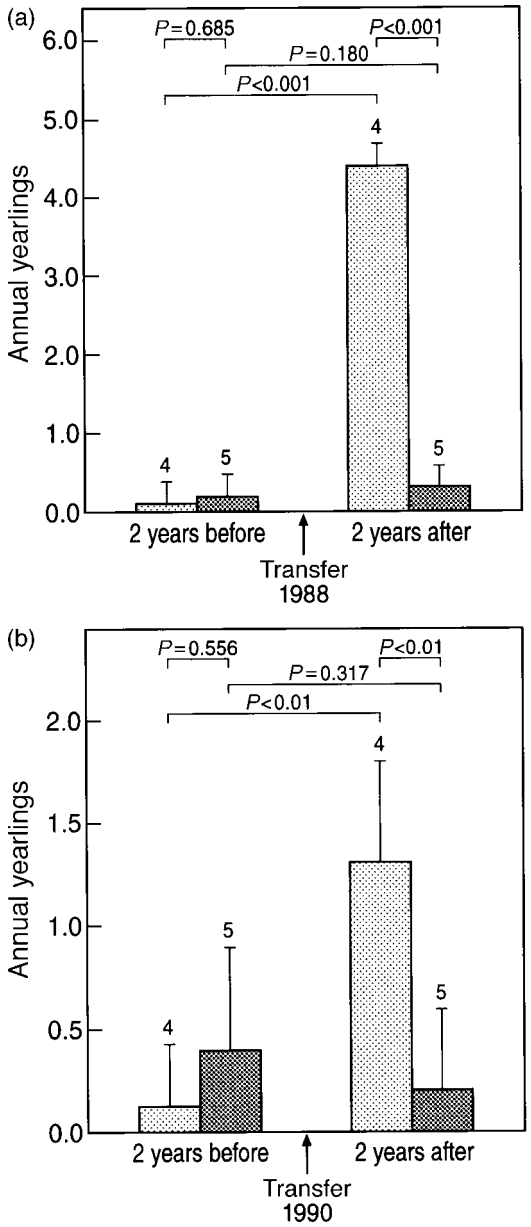

Fig. 5. Annual number of yearlings produced per experimental (open bars) and control (shaded bars) pairs of Seychelles warblers two years before (Aride: 1986 to 1988; Cousine: 1988 to 1990) and two years (1988 to 1990) or one year (1990 to 1991) after the transfer to the islands of (a) Aride in 1988 and (b) Cousine in 1990, Means, standard deviations and sample sizes are shown. P values for differences between experimental and control groups were determined by one-tailed Mann–Whitney U test; those for pre- and post-removal were determined by one-tailed paired-sample t test.

The increase in reproduction by the experimental groups on Aride was attributed to more successful breeding attempts per year (Table 2): (1) higher number of nesting attempts per pair; (2) higher survival of clutches to hatching, and consequently higher number of nests with nestlings and nests producing fledglings; (3) higher survival of fledglings to one year of age, and consequently higher number of one year old young produced per pair. The increase in reproduction was not only due to more successful breeding attempts per year, but also to a higher success per nest (Table 2). This was attributed to: (1) a larger clutch size; (2) higher hatching success, and as a consequence more nestlings per nest; (3) higher nestling survival to fledgling, and more fledglings per nest, and (4) higher fledgling survival to one year of age. Taken together, the production of yearlings per experimental pair was, on average, 44.0 times higher on Aride. The higher breeding output is in agreement with the fact that the mean territory quality of experimental pairs on Aride was 14.5 times higher than that on Cousin (Table 2).

The increase in reproduction by the experimental groups on Cousine was attributed to the higher number of nests built per pair, not (as on Aride) to higher nesting success. The percentage of nests with clutch, clutch size, hatching and fledging success were all slightly, but not significantly, higher on Cousine than on Cousin. In addition, fledgling survival to one year of age was five times higher on Cousine. Taken together, these factors resulted in a five times higher production of yearlings by the experimental pairs. Mean territory quality of experimental pairs on Cousine was 3.4 times higher than that on Cousin (Table 2). These results are in agreement with the fact that the quality of warbler territories was intermediate compared to that of Aride and Cousin (Table 2).

The increased breeding on Aride and Cousine was not due to temperature and rain levels that were above a certain threshold level during part or all of the year. Mean monthly temperature, monthly rainfall and monthly rainfall frequency of these islands (Table 2) as well as their annual patterns (Komdeur, 1994*a*, 1996*a*) were the same as for Cousin (Table 2). On Aride in 1996, eight years after the transfer, the population had grown to over 1000

Table 2. Comparison of mean annual breeding performance, mean monthly territory quality and mean monthly weather variables of four Seychelles warbler pairs (± SD) on Cousin Island before (October 1986–September 1988) and on Aride Island after translocation (October 1988–September 1990), and of four pairs on Cousin before (July 1988–June 1990) and on Cousine Island after translocation (July 1990–July 1991)

	Experimental groups (n = 4)			Experimental groups (n = 4)		
	Cousin (1986–1988)	Aride (1988–1990)	P	Cousin (1988–1990)	Cousine (1991–1991)	P
Annual performance/pair:						
Nest attempts	1.5 ± 0.6	5.1 ± 0.5	<0.001	1.0 ± 0.7	2.3 ± 1.3	<0.025
Nests with clutch (%)	83.5	92.7	0.680	100.0	81.3	0.506
Nests with clutch	1.3 ± 0.3	4.8 ± 0.4	<0.001	1.0 ± 0.7	1.8 ± 1.0	<0.025
Clutches hatched (%)	50.0	94.7	<0.002	50.0	79.2	0.751
Nests with nestlings	0.6 ± 0.3	4.5 ± 0.0	<0.001	0.5 ± 0.4	1.3 ± 0.5	<0.025
Nestlings fledged (%)	80.0	97.2	0.570	50.0	100.0	0.906
Nests producing fledglings	0.5 ± 0.4	4.4 ± 0.3	<0.001	0.4 ± 0.5	1.3 ± 0.3	<0.01
Fledglings reaching one year of age (%)	25.0	100.0	<0.001	12.5	100.0	<0.05
Nests producing yearlings	0.1 ± 0.3	4.4 ± 0.3	<0.001	0.13 ± 0.3	1.3 ± 0.5	<0.01
Fate of clutch:						
Clutch size (n)	1.0 ± 0.0 (10)	1.8 ± 0.1 (36)	<0.001	1.0 ± 0.0 (6)	1.1 ± 0.4 (7)	0.731
Eggs hatched (%) (n)	50.0 (10)	83.1 (65)	x0.05			
Nestlings hatched/nest (n)	0.5 ± 0.3 (10)	1.5 ± 0.2 (36)	<0.05			
Nestlings fledged (%) (n)	80.0 (5)	100.0 (65)	<0.02			
Nestlings fledged/nest (n)	0.4 ± 0.4 (10)	1.5 ± 0.2 (36)	<0.05			
Fledglings reaching one year of age (%) (n)	25.0 (4)	100.0 (65)	<0.02			
Yearlings produced/nest (n)	0.1 ± 0.3 (10)	1.5 ± 0.2 (36)	<0.002			
Mean territory quality	17.8 ± 10.3	257.8 ± 45.0	<0.002	8.5 ± 6.0	28.9 ± 7.4	<0.025
Mean monthly temperature (°C)	30.2 ± 1.4	30.5 ± 1.8	0.913	30.8 ± 1.2	30.5 ± 1.4	0.861
Main monthly rainfall (mm)	140.2 ± 60.1	121.8 ± 98.2	0.645	132.4 ± 57.2	135.2 ± 45.9	0.913
Mean monthly rainfall frequency (days)	13.1 ± 2.3	15.2 ± 2.4	0.752	16.8 ± 3.1	17.5 ± 2.9	0.873

Figures in parentheses are number of observations. Statistical significance of comparisons between annual performance before and after translocation determined by two-tailed Wilcoxen test (percentage data) or two-tailed paired sample t test. Statistical significance of comparisons between fate of clutch before and after translocation determined by chi-squared contingency analysis (percentage data) or two-tailed t test. Statistical significance of comparisons between mean monthly territory quality and mean monthly weather variables before and after translocation determined by two-tailed t test.

individuals (Fig. 1), and 12 transferred birds were still alive. On Cousine in the same year, six years after the transfer, the population had grown to 186 individuals (Fig. 1), and 10 transferred birds were still alive.

DISCUSSION

Bottlenecks and genetic variation

The Seychelles warbler population is probably an inbred population and has lost a considerable amount of genetic variation, as compared with the average of outbred natural bird populations (Burke & Bruford, 1987; Wetton, Carter *et al.*, 1987; Westneat, 1990; Meng, Carter & Parkin, 1990). The warbler population on Cousin supposedly did suffer from considerable genetic erosion during the known nine-year bottleneck 25 years ago, or during a possible decline of population size due to destruction of vegetation since 1910 (Collar & Stuart, 1985), or even earlier at the time warblers initially colonized Cousin. Similar results were found for the Scandinavian population of Canada geese (*Branta canadensis*) that went through an even more extensive bottleneck during the last century. Only five individuals were released in Sweden and have increased to a current population size of around 30–50 000 birds (Fabricius, 1983; Madsen & Andersson, 1990). This rapidly expanding population of Canada geese also is pauperized with respect to genetic variation as witnessed by a band-sharing coefficient of 0.76, which is even higher than that of the Seychelles warblers (Tegelström & Sjöberg, 1995). On the other hand, small isolated populations can still have a high level of genetic variation and a high reproduction rate, as was shown for a colony of 39 Waldrapp ibises (*Geronticus eremita*). Despite the fact that the colony was founded by only six birds and that some of the founders were related, DNA fingerprinting revealed highly polymorphic banding patterns, indicating a high level of genetic variation (Signer, Schmidt & Jeffreys, 1994). These facts illustrate that the initial level of genetic variation may be of great importance for the maintenance of genetic diversity during one or more bottlenecks. Unfortunately, we cannot make a proper comparison between the levels of genetic variation of the Cousin population before and after the bottleneck, because the level of genetic variation before the bottleneck is unknown. However, given the low band-sharing coefficient of the Australian warbler, its closest mainland relative (Leissler *et al*., 1997), we surmise that the level of genetic variation in the Seychelles warbler before the bottleneck was higher. When individual Seychelles warblers with known relatedness of $r = 0.5$ are compared, a band-sharing coefficient of 0.64 ± 0.12 and 0.65 ± 0.10 is observed for the Cousin and Aride population, respectively (A. Kappe, unpublished data). Thus, the amount of genetic variation in the Seychelles warbler is not completely exhausted. This may be due to the fact that there has been selection against inbred Seychelles warblers during the past stressful conditions, as has been described for song sparrows (*Melospiza*

melodia; Keller *et al.*, 1994). Interestingly, the reproductive success of pairs comprising related birds (r = 0.5) did not differ significantly from that of pairs comprising unrelated birds. This may indicate that some effective purging has occurred during either the colonization process and/or the recent bottleneck period.

Heterozygosity and viability

It is not completely known how the level of genetic variation relates to a population's persistence in the long term. Merola (1994) argued that genetic uniformity as present in the cheetah (*Acinonyx jubatus*), does not necessarily appear to compromise the survival chances of the species, at least not in the short term. Likewise, despite the relatively high degree of inbreeding, reproductive success of the small group of Seychelles warblers on the previously unoccupied islands of Aride and Cousine was high. These differences in reproductive success of the warblers on the three islands are not due to gross genetic differences, but mainly to differences in environmental conditions. On Aride and Cousine, with the same vegetation and climatical conditions, the abundance of insect food per territory is significantly higher than on Cousin. The survival of progeny and the level of individual fitness of warblers on Aride and Cousine are high, resulting in an extremely high population growth rate leading to a population size of more than 1000 birds on Aride and 186 on Cousine in 1996. However, we expect that in the future the breeding success will decline and eventually become the same as that of warblers on Cousin because: (1) the growing warbler population may reduce insect food levels on the island; (2) with increasing degree of saturation of the island with warbler territories, territories may become smaller leading to lowered food abundance per territory.

Low levels of genetic variation are associated with the occurrence of inbreeding depression, i.e. the deterioration of fitness characters (Chambers, 1983; Bensch, Hasselquist & von Schantz, 1994). Despite the relatively low levels of genetic variation in the Seychelles warbler populations, no apparent deleterious effects have yet been reported, though of course, there are no reference populations containing the original level of genetic variation. It is possible that the population has been purged of genetic load by previous inbreeding periods, including the nine-year bottleneck. However, as was demonstrated by Hedrick (1994), the mean fitness of the population may be reduced due to the fixation process, so that the probability of extinction in the long term is increased. The population on Aride apparently encounters luxurious amounts of insects, compared to the Cousin situation. This highly advantageous situation may conceal the potentially high risks of inbreeding, which will be exhibited during less favourable conditions. A possible approach to assess the adaptive potential of the Seychelles warbler would be a translocation to an island with significantly different conditions (with respect to insect abundance, vegetation, parasites) or translocation to an island of inbred and less inbred pairs. Despite the absence of a demonstrable deleterious effect, the recent bottleneck may have a negative influence on the future of the Seychelles warbler populations.

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